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Clin. Vaccine Immunol. 2006, 13(3):361. DOI:
10.1128/CVI.13.3.361-364.2006.

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Evaluation of Vircell Enzyme-Linked Immunosorbent Assay and Indirect Immunofluorescence Assay for Detection of Antibodies against *Legionella pneumophila*

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Received 3 August 2005/Returned for modification 13 September 2005/Accepted 13 January 2006

We evaluated the abilities of the Vircell immunoglobulin G (IgG) and IgM indirect immunofluorescence assay (IFA) for *Legionella pneumophila* serogroup 1, the IgM and IgG enzyme-linked immunosorbent assay (ELISA) for *Legionella pneumophila* serogroup 1, and the IgM-plus-IgG ELISA for *Legionella pneumophila* serogroups 1 to 6 to diagnose Legionnaires' disease (LD) in a well-described sample of patients with and without LD. Also, we determined the agreements, sensitivities, and specificities of the different Vircell assays in comparison to a validated ELISA (Serion classic ELISA). Clinical sensitivity and specificity were 74.6% and 96.6%, respectively, for the IgM IFA, 65.1% and 88.0% for the IgG IFA, 92.3% and 100% for the IgM ELISA, 43.3% and 96.6% for the IgG ELISA, and 90.8% and 100% for the IgM-plus-IgG ELISA. Compared to Serion classic ELISA, agreement, sensitivity, and specificity were 80.0%, 83.1%, and 78.4%, respectively, for the IgM IFA, 75.2%, 66.0%, and 79.5% for the IgG IFA, 89.5%, 82.0%, and 97.6% for the IgM ELISA, 81.9%, 88.9%, and 78.0% for the IgG ELISA, and 93.5%, 90.0%, and 96.6% for the IgM-plus-IgG ELISA. The value of a positive diagnostic result obtained by the Vircell IgM IFA, the Vircell IgG IFA, and the Vircell IgG ELISA might not be acceptable for a diagnostic assay. Both the high specificities and sensitivities of the Vircell IgM ELISA and the IgM-plus-IgG ELISA and the high correlation with the Serion classic ELISA indicate that they are useful in the diagnosis of LD.

The genus *Legionella* of the family *Legionellaceae* includes more than 45 species of fastidious gram-negative bacilli, 20 of which have been reported to infect humans. Bacteria of the family *Legionellaceae* are ubiquitous in both natural and man-made aqueous environments, and inhalation or aspiration of contaminated water can cause Legionnaires' disease (LD), a severe pneumonia. Legionellae can also cause subclinical infection and extrapulmonary inflammation (5). *Legionella pneumophila* causes 91% of all reported cases of LD, with serogroup 1 being the most predominant serogroup, causing approximately 80% of all culture-confirmed cases (10).

Of the various antibody detection methods that are available to detect *Legionella* infection, indirect immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays (ELISA) are the most commonly used methodologies (1, 3, 8). A fourfold or greater increase in the titer of antibody is considered diagnostic. An ELISA is generally preferred over IFA testing because it is less subjective, is thought to be more sensitive than IFA testing, and has the potential for automated performance (1, 7, 14). The availability of commercial ELISA and IFA kits has resulted in the increasing use of these products, despite the fact that few studies determining their sensitivity and specificity are available.

The aim of our study was to evaluate commercial ELISA and

IFA (Vircell, S.L., Santa Fé, Granada, Spain) for the detection of antibodies against *L. pneumophila*.

MATERIALS AND METHODS

Clinical samples. Serum samples were collected between 1999 and 2004 and stored at -20°C until processing was performed. We included 129 serum samples of 65 patients with proven LD (cases). A proven case of LD was defined as a patient who suffered from symptoms of pneumonia, had radiological signs of infiltration, and showed laboratory evidence of infection with *L. pneumophila*. Laboratory evidence included one or more of the following criteria: isolation of *L. pneumophila* from a respiratory sample; a positive urinary antigen test (Binax Now *Legionella* urinary antigen test; Binax Inc.); a positive PCR result on respiratory tract samples using a 16S rRNA assay (12); a single high titer in immunoglobulin M (IgM) and/or IgG; and/or seroconversion to positive IgM and/or IgG antibodies to *L. pneumophila* (*L. pneumophila* antibodies of serogroups 1 to 7) in paired acute-phase and convalescent-phase sera using a commercial enzyme-linked immunosorbent assay (Serion ELISA; Institut Virion/Serion GmbH, Würzburg, Germany). The Serion ELISA has more or less been validated in extensive studies (1, 2). Age and sex distributions of cases were as follows: 46 were male, and 19 were female, with ages between 32 and 79 years (mean age, 54.5). The laboratory results of patients with proven LD (number testing positive/number tested) were as follows: serology, 65/65 (100%); culture on respiratory tract samples, 14/28 (50%); PCR on respiratory tract samples, 24/24 (100%); and urinary antigen test, 52/58 (90%).

In addition, serum samples of patients with respiratory tract infections other than *Legionella* were tested in a similar manner to serve as controls and to test specificity. These samples were obtained from 29 patients with respiratory tract infections and who had a fourfold or greater increase in (complement-fixating) antibodies against influenza A virus, adenovirus, *Chlamydia psittaci*, or *Mycoplasma pneumoniae* (50 samples). Age and sex distributions of controls were as follows: 15 were male, and 14 were female, with ages between 2 and 84 years (mean age, 43.5).

***Legionella pneumophila* IgM and IgG IFA (Vircell, S.L., Santa Fé, Granada, Spain).** Samples were tested for *L. pneumophila* serogroup 1 IgM and IgG antibodies by an IFA according to the manufacturer's instructions (11). IgG

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TABLE 1. Clinical sensitivity and specificity obtained using Vircell IFA and Vircell ELISA for detection of *Legionella pneumophila* IgM- and IgG-specific antibodies in patients with and without LD^a

Parameter	No. (%) of positive samples/total no. tested				
	IgM IFA	IgG IFA	IgM ELISA	IgG ELISA	IgM-plus-IgG ELISA
Sensitivity	47/63 (74.6)	41/63 (65.1)	60/65 (92.3)	26/60 (43.3)	59/65 (90.8)
Specificity	1/28 (3.6)	3/25 (12.0)	0/29 (0)	1/29 (3.5)	0/27 (0)

^a Samples were tested for *L. pneumophila* serogroup 1 antibodies in an IgM IFA, IgG IFA, IgM ELISA, and IgG ELISA and *L. pneumophila* serogroups 1 to 6 in an IgM-plus-IgG ELISA.

titers of $\geq 1:64$ or IgM titers of $\geq 1:96$ as well as seroconversion (fourfold rise in titer) were considered positive.

Vircell IgM, IgG, and IgM-plus-IgG ELISA (Vircell, S.L., Santa F , Granada, Spain) for *Legionella pneumophila* serogroups 1 to 6. The ELISA kits included a microplate coated with lipopolysaccharide from *Legionella pneumophila* from either serogroup 1 (IgM, IgG) or serogroups 1 to 6 (IgM plus IgG). For IgM testing, IgG and rheumatoid factor were removed by treatment with sorbent directly into the well. Samples were tested, and the results were interpreted in accordance with the package inserts provided with the assays.

Serion classic ELISA of *L. pneumophila* serogroups 1 to 7 (Serion Immunodiagnostica, W rzburg, Germany). Samples were tested for IgG and IgM antibodies against *Legionella pneumophila* serogroups 1 to 7. Samples were tested, and the results were interpreted in accordance with the package inserts provided with the two assays.

Statistical analysis. Clinical sensitivity and specificity of the assays were determined using a two-by-two contingency table. Also, the agreements, sensitivities, and specificities of the Vircell assays were determined by comparing the results with those obtained with the Serion classic ELISA. Equivocal results were not included in the calculations.

RESULTS

Clinical sensitivity and specificity were 74.6% and 96.6%, respectively, for the IgM IFA and 65.1% and 88.0% for the IgG IFA (Table 1). If the results of both the IgM IFA and IgG IFA of each separate assay were combined, clinical sensitivity and specificity would be 87.5% and 84.0%, respectively. Clinical sensitivity and specificity were 92.3% and 100%, respectively, for the IgM ELISA, 43.3% and 96.6% for the IgG ELISA, and 90.8% and 100% for the IgM-plus-IgG ELISA (Table 1). If the results of both the IgM ELISA and IgG ELISA of each separate assay were combined, clinical sensitivity and specificity would be 91.1% and 96.6%, respectively.

A calculated agreement, sensitivity, and specificity of 80.0%, 83.1%, and 78.4%, respectively, were found for the IgM IFA compared to the Serion IgM ELISA and 75.2%, 66.0%, and 79.5%, respectively, for the IgG IFA compared to the Serion IgG ELISA (Table 2). Of the 179 samples tested, 23 (9 in the IgM IFA and 14 in the IgG IFA) were equivocal results that were not included in the calculations.

A calculated agreement, sensitivity, and specificity of 89.5%, 82.0%, and 97.6%, respectively, were found for the Vircell IgM ELISA compared to the Serion IgM ELISA and 81.9%, 88.9%, and 78.0%, respectively, for the Vircell IgG ELISA compared to the Serion IgG ELISA (Table 3). A calculated agreement, sensitivity, and specificity of 93.5%, 90.0%, and 96.6%, respectively, were found for the Vircell IgG-plus-IgM ELISA compared to the Serion IgM ELISA. Of the 179 samples tested, 31 (8 in the IgM ELISA, 13 in the IgG ELISA, and 10 in the IgG-plus-IgM ELISA) were equivocal results that were not included in the calculations.

DISCUSSION

Several methods of antibody detection against *L. pneumophila* have been developed, such as IFA, microagglutination test, indirect hemagglutination test, and ELISA (1, 6, 7, 9, 13, 15). Historically, the IFA was the first assay used to detect antibodies against *Legionella pneumophila*. Nowadays, commercially available ELISA kits are extensively used for the diagnosis of LD because they are at least as sensitive as IFA, can have an objectively determined end point, and allow automation. The reported sensitivities of serological assays vary substantially, from 41% to 94% (1, 3, 4, 6). This variation may be due to differences in the study population, the design of the study, differences in the antigen preparation or in the valence (mono- or polyvalent) of the antigen used, cross-reacting antibodies, and differences in the ability to detect IgM or IgG. In this study, we evaluated the ability of commercial IFA and ELISA to diagnose LD in a well-described population of patients with and without LD. Also, we determined the agreements, sensitivities, and specificities of the different Vircell assays in comparison to a validated ELISA (1, 2).

The clinical sensitivity of the Vircell ELISA to diagnose LD was significantly higher for the detection of IgM and IgM-plus-IgG antibodies in comparison to that of the IFA for IgM detection (92.3%, 90.8%, and 74.6% for the Vircell IgM ELISA, the IgM-plus-IgG ELISA, and the IgM IFA, respectively; $P < 0.01$). The clinical sensitivity of the Vircell ELISA for the detection of IgG-specific antibodies was significantly lower than that of the IFA for IgG detection (43.3% and 65.1% for the Vircell IgG ELISA and the IgG IFA, respectively; $P = 0.02$).

TABLE 2. Agreement, sensitivity, and specificity of Vircell *Legionella* IFA compared to Serion classic ELISA for detection of *L. pneumophila* IgM- and IgG-specific antibodies^a

Serion ELISA result	No. of samples ^b					
	Positive		Negative		Equivocal	
	IgM IFA	IgG IFA	IgM IFA	IgG IFA	IgM IFA	IgG IFA
Positive	49	35	24	23	5	4
Negative	10	18	87	89	2	6
Equivocal	2	2	0	2	0	0

^a Samples were tested for *L. pneumophila* serogroup 1 antibodies in an IgM IFA, IgG IFA, IgM ELISA, and IgG ELISA and *L. pneumophila* serogroups 1 to 6 in an IgM-plus-IgG ELISA.

^b Agreement, sensitivity, and specificity were 80.0%, 83.1%, and 78.4%, respectively, for the IgM IFA compared to the Serion IgM and 75.2%, 66.0%, and 79.5%, respectively, for the IgG IFA compared to the Serion IgG.

TABLE 3. Agreement, sensitivity, and specificity of Vircell *Legionella* ELISA compared to Serion classic ELISA for detection of *L. pneumophila* IgM- and IgG-specific antibodies^a

Serion ELISA result	No. of samples ^b								
	Positive			Negative			Equivocal		
	IgM ELISA	IgG ELISA	IgM + IgG ELISA	IgM ELISA	IgG ELISA	IgM + IgG ELISA	IgM ELISA	IgG ELISA	IgM + IgG ELISA
Positive	73	32	72	2	26	3	1	3	1
Negative	16	4	8	80	104	86	5	2	7
Equivocal	2	4	2	0	4	0	0	0	0

^a Samples were tested for *L. pneumophila* serogroup 1 antibodies in an IgM IFA, IgG IFA, IgM ELISA, and IgG ELISA and *L. pneumophila* serogroups 1 to 6 in an IgM-plus-IgG ELISA.

^b Agreement, sensitivity, and specificity were 89.5%, 82.0%, and 97.6%, respectively, for the Vircell IgM compared to the Serion IgM; 81.9%, 88.9%, and 78.0%, respectively, for the Vircell IgG compared to the Serion IgG; 93.5%, 90.0%, and 96.6%, respectively, for the Vircell IgM and IgG compared to the Serion IgM.

Compared to that of the Serion classic ELISA, the sensitivity of the Vircell ELISA was higher for the detection of IgM antibodies and IgM-plus-IgG antibodies but not statistically significant (92.3%, 90.8%, and 87.5% for the Vircell IgM ELISA, the IgM-plus-IgG ELISA, and the Serion classic ELISA, respectively). The sensitivity to detect IgG was significantly lower for Vircell in comparison to that of Serion (44.0% versus 76.5% for Serion; $P < 0.001$).

The correlation between Serion and Vircell varied between the assays evaluated. The correlation was highest between the Serion classic ELISA and the Vircell ELISA for the detection of IgM and IgG combined and lowest for the IgG IFA (agreement, sensitivity, and specificity of 93.5%, 90.0%, and 96.6% for the Vircell ELISA and 75.2%, 66.0%, and 79.5% for the IgG IFA). Although equivocal results were not included in the calculations, a considerable percentage of samples gave equivocal results in both the Vircell IFA and ELISA. For example, in the IgM IFA, 6.4% (5/78) of samples positive in the Serion ELISA tested equivocal. Similar percentages can be seen for both the Vircell ELISA and IFA.

A drawback in the current evaluation is that we evaluated a relatively small group of patients with respiratory tract infections other than LD. This could influence the specificity. Also, the majority of LD-positive patients were infected with *L. pneumophila* serogroup 1, making it difficult to conclude anything for *L. pneumophila* infections caused by other serogroups.

Usually both IgM and IgG can be detected in samples of patients during the course of their illness, but in a significant proportion of patients, no IgG is detectable (6). Studies have shown that many patients produce primarily IgM antibodies and that these are useful for the early diagnosis of Legionnaires' disease (5, 11). However, IgM may be present later in some confirmed cases, limiting the usefulness of the assay for early diagnosis in all patients. In a study by Rojas et al., serum antibody detection (Vircell) and urine antigen detection techniques (Binax Now) were compared in samples from 116 patients epidemiologically characterized as belonging to a legionellosis outbreak (11). ELISA for IgM, ELISA for IgG plus IgM, antigenuria detection, and IFA for IgM were able to diagnose 72.3%, 60.5%, 53.3%, and 51.4% of patients, respectively. Antigenuria was detected in 53.8% of first samples, ELISA detected IgM in 29.7%, ELISA detected IgG plus IgM in 7.9%, and IFA detected IgM in 3.9%. For the patient series as a whole, serological techniques displayed greater sensitivi-

ties than antigen detection; 35 patients would have been scored as negative if serological testing had not been carried out.

For an illness of low prevalence, such as LD, the specificity of a test is an important parameter. Our results suggest that the predictive value of a positive diagnostic result obtained by the Vircell IgM IFA, the Vircell IgG IFA, and the Vircell IgG ELISA might not be acceptable for a diagnostic assay. The positive and negative predictive values obtained by the Vircell IgM ELISA and the IgM-plus-IgG ELISA approach 100%, assuming that the prevalence of LD is 4% of patients with pneumonia. Both the high sensitivities and specificities of these two assays and their high correlation with the Serion classic ELISA indicate that considerable confidence can be placed in the validity of the diagnosis when specimens from a patient give serodiagnostic results.

ACKNOWLEDGMENT

We thank Vircell, S.L. (Santa Fé, Granada, Spain), for supplying the assays evaluated in this study.

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AUTHOR'S CORRECTION

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Volume 13, no. 3, p. 361–364, 2006. Page 362: Table 2 should appear as shown below.

TABLE 2. Comparison of the Vircell *Legionella* IFA to the Serion classic ELISA for detection of *L. pneumophila*-specific IgM and IgG antibodies^a

Serion ELISA result	No. of samples tested by Vircell <i>Legionella</i> IFA ^b					
	Positive		Negative		Equivocal	
	IgM	IgG	IgM	IgG	IgM	IgG
Positive	47	35	24	24	5	2
Negative	11	17	88	88	2	5
Equivocal	2	4	0	3	0	1

^a Samples were tested for antibodies specific for *L. pneumophila* serogroup 1 in IFAs for IgM and IgG as well as in ELISAs for IgM and IgG and for antibodies specific to *L. pneumophila* serogroups 1 to 6 in an ELISA for both IgM and IgG. Results presented are compiled from the individual and combined ELISAs.

^b For IFA compared to ELISA, the agreement, sensitivity and specificity were, respectively, 79.4%, 66.2%, and 88.9% for IgM and 75.0%, 59.3%, and 83.8% for IgG.

Page 363, Table 3: Footnote *b* should read as follows. “Agreement, sensitivity, and specificity were 89.5%, 97.3%, and 83.3%, respectively, for the Vircell IgM ELISA; 81.9%, 55.2%, and 96.3%, respectively, for the Vircell IgG ELISA; and 93.5%, 96.0%, and 91.5%, respectively, for the Vircell IgM and IgG combined ELISA.”